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Reversal of the Effects of Aging in Soybean Seeds[1]

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ABSTRACT

Accelerated aging predisposed seeds to imbibition injury. Slowing the rate of hydration prevented the loss of germinability due to imbibition injury. Germinability of accelerated aged seeds (50 hours) was increased from 10 to 90% by controlling the rate of imbibition. Slow hydration also prevented seed electrolyte leakage. This may indicate that cell membrane permeability or rupture was a major factor contributing to the loss of germinability after aging.

Reversal of the effects of aging (repair) was accomplished by slowly inbibing and then redrying seeds (priming). This treatment lowered steep water conductivity by a factor of 2 to 5. Priming also increased the per cent germination of low vigor seeds. The mechanism of this reversal was probably metabolic because it depended on temperature, seed moisture, and treatment duration.

Priming doubled the survival of seeds in the accelerated aging vigor test. The 'rejuvenation' was accepted as evidence for metabolic repair. Since the 'vigor' of seeds was increased by priming, metabolic repair probably included other subcellular components as well as the plasma membrane.

Repair during germination is a hypothetical event supported by some investigators and questioned by others. Repair includes the spontaneous reorganization of plasma membrane phospholipids, which according to Simon (2) occurs during seed hydration. Alternatively or additionally, metabolic repair might occur, as suggested from the work of Toole and Toole (4), Villiers (5), and Villiers and Edgecumbe (6).

Experimental evidence for repair in imbibed seeds has been elusive. The objectives of this study and of a more detailed report (3) are to test for the existence of repair mechanisms and to determine if these are spontaneous or metabolic.

MATERIALS AND METHODS

Seed Material. Locally grown soybean seeds (*Glycine max* [L] Merr. cv Vicoja) were used in all experiments. Seeds stored in an air-conditioned room were equilibrated to 11% on a fresh weight basis. Stored seeds were also coated with a powder containing Captan and Botran fungicides.

Accelerated Aging. Standard equipment (Stults Scientific Engineering Corp., Springfield, IL) and conditions (41°C, 100% RH) were used for accelerated aging (1). Precautions ensured seeds absorbed moisture as water vapor and not as liquid water resulting from condensation. Accelerated aging treatments were 20, 30, 40, and 50 h. During aging, seed moisture increased from 11% to a maximum of 30%. After accelerated aging, the seeds were re-equilibrated to 11% moisture by air-drying them for 3 d in ambient conditions.

Controlled Water Uptake. The rate of seed hydration was controlled by adding multiple layers of germination paper (Anchor, Ann Arbor) to a constant quantity of water. Deionized water (20 ml) was placed in Petri dishes (25 × 150 mm), then in progression, one paper disk was placed in the first dish and five were placed in the fifth dish; 20 seed were added to each dish. Orientation of the seeds on the paper limited contact to one cotyledon, avoiding direct imbibition by the axes. Imbibition rate was monitored by weighing the seeds at 1-, 3-, 6-, 20-, and 28-h intervals.

Measurement of Electrolytes. After soaking seeds for 8 to 10 h, an automatic seed analyzer (ASA-610, Agro Sciences, Inc.) was used to monitor leakage from them. Some experiments

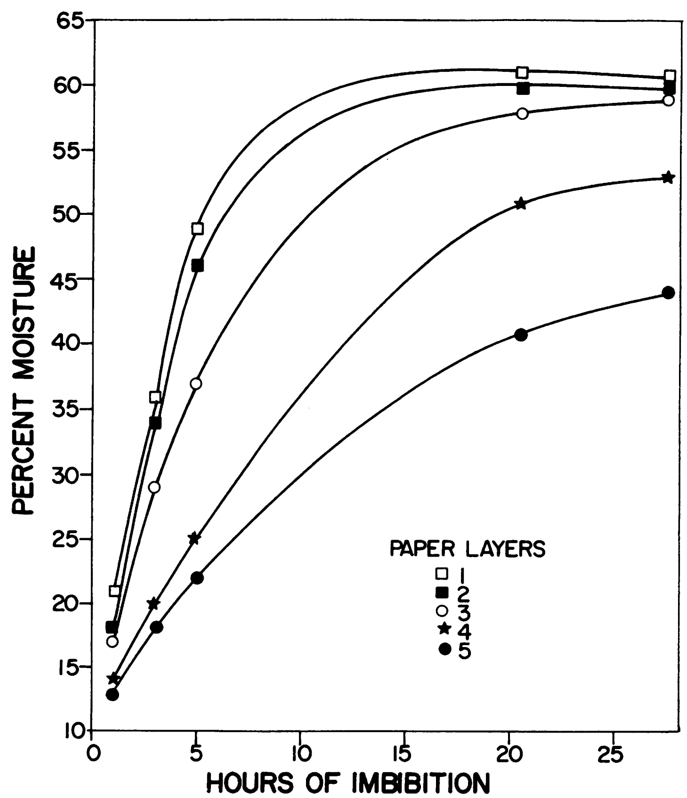

FIG. 1. Soybean seeds with an initial moisture of 11% were imbibed in five Petri plates (25 × 150 mm), each containing 20 ml of deionized H_2O. Each plate also contained 20 seeds and 1 to 5 layers of germination paper (one in the first plate and five in the fifth). The seeds in each plate were weighed after 1, 3, 6, 20, and 28 h of inbibition. The seed moisture was expressed on the fresh weight basis.

[1] This work represents a portion of research submitted by Robert L. Tilden to the University of Florida in partial fulfillment of the requirements for the Ph.D. degree.

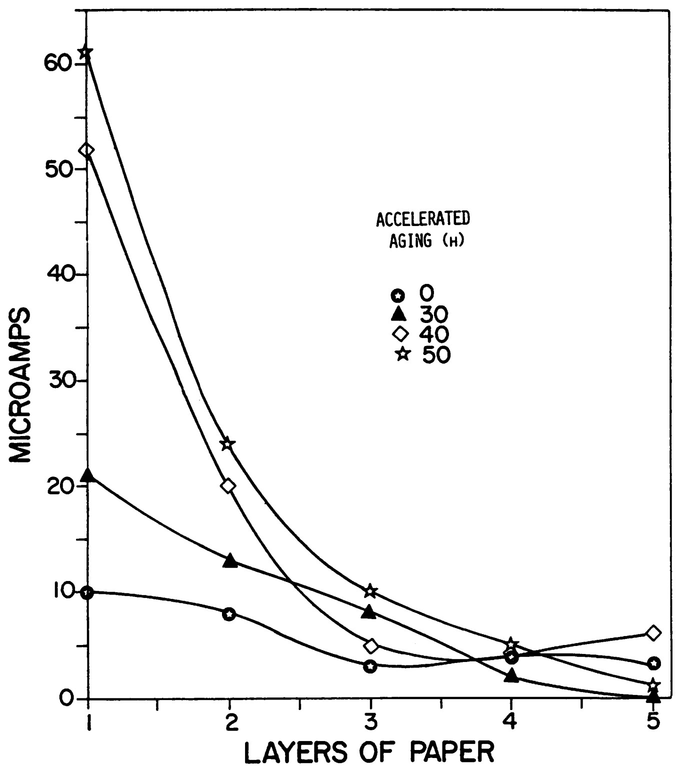

FIG. 2. The conductivity of electrolytes recovered from imbibition papers as a function of both seed aging (0–50 h) and five rates of water uptake (described in "Materials and Methods"). Each point (μamps) is the average of four replicated aliquots taken from 60 ml of leached solution.

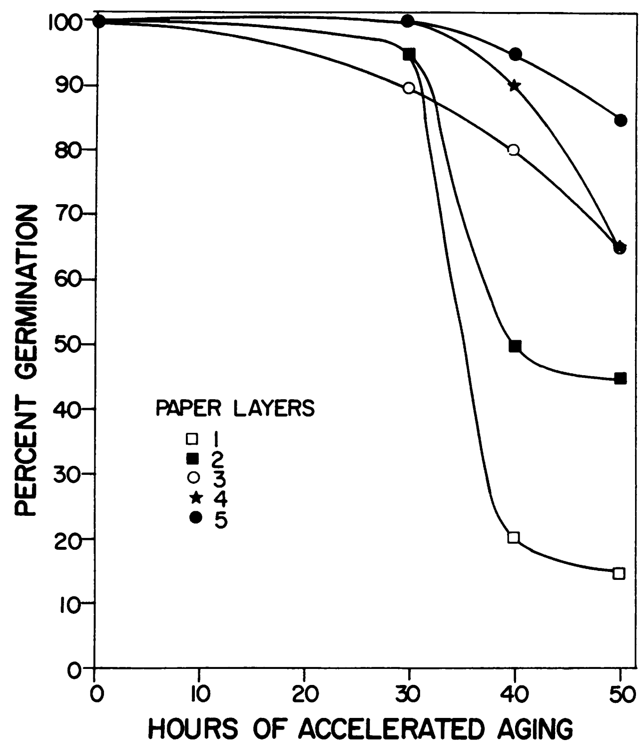

FIG. 3. The loss of seed vitality (per cent germination) resulting from accelerated aging for five rates of imbibition. Aging and control of imbibition are described in "Materials and Methods".

measured seed leakage indirectly by leaching the papers used for imbibition with 60 ml of water. Four-ml aliquots of the steep solution were then placed in the ASA-610 for conductivity measurements (μamp). Corrections were made for the electrolytes contained in the germination paper.

Priming. Seeds were primed by slowly imbibing them (four layers of germination paper, 20 ml water) in Petri dishes (150 mm diameter). After 2 d at 25°C, the seeds were allowed to air-dry by uncovering the Petri dishes for 4 d, also at 25°C, unless otherwise indicated.

Germination and Growth. Seeds were germinated in the dark at 25°C on two germination papers and 20 ml of water. Twenty seeds were germinated in each Petri plate. After 5 d of growth, the fresh weight and root-hypocotyl weights of all seeds were recorded for each test plate. Each test (observation) was replicated at least twice.

RESULTS

Controlled Water Uptake. Figure 1 illustrates that the rate of seed hydration was effectively slowed by each additional layer of germination paper. The maximum seed moisture was 61%. Approaching this upper limit, the curves that describe seed moisture are a function of the log of time: ($\%M = I + C{*}\log$ [time]). Using a least squares linear regression analysis, the coefficient of the determination (R-square) was typically 0.95. The intercept (I) and coefficient (C) were functions of the number of papers.

Avoidance of Imbibitional Injury. The rate at which seeds imbibed water was controlled by varying the number of layers of paper in the Petri dish. As a result of aging, rapid uptake (one or two layers of paper) resulted in copious electrolyte leakage (Fig. 2). As the uptake was slowed, there was a corresponding reduction in electrolytes recovered from the imbition papers, such that the amount lost by aged seeds was reduced to that of unaged seeds.

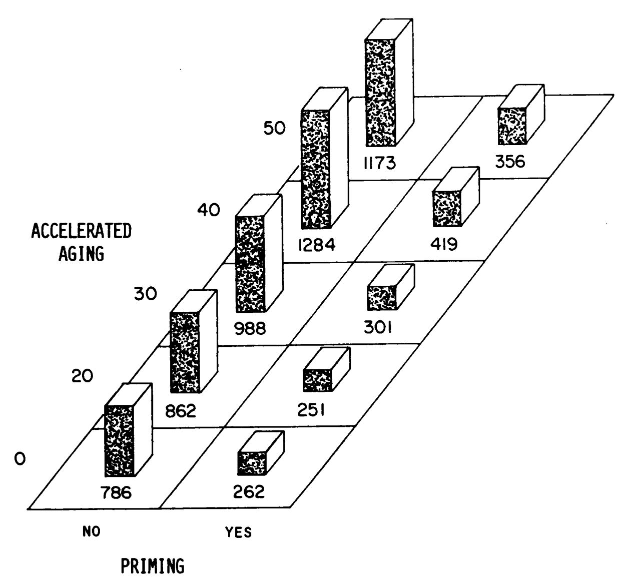

FIG. 4. Effect of priming on electrolyte leakage. A factorial experiment consisted of five levels of accelerated aging (0, 20, 30, 40, and 50 h) with and without priming. All seeds were equilibrated back to 11% moisture before the electrolyte leakage test. Conductivity measurements were collected after 9 h of soaking, including imbibition, and expressed as the sum of the microamperes of the electrolytes released by each of the 20 seeds in each of the 10 treatments.

Prevention of imbibition injury also prevented loss of seed

Table I. *Effect of Priming Temperature on the Electrolyte Leakage of Accelerated Aged and Unaged Soybean Seeds*

Treatment Temp		Results							
		Aged				Unaged[a]			
Wet[b]	Dry[c]	Rank[d]	ASA[e]	SD[f]	% of control	Rank	ASA	SD	% of control
°C		μamps/seed·4 ml							
4	4	1	85	8.6	111	1	68	12.9	113
4	25	2	57	0.9	74	2	50	3.7	84
25	4	3	50	8.6	65	3	38	9.2	64
25	25	4	47	4.7	61	4	28	2.9	47

[a] Unaged seeds had been stored for 1 year (65% RH, 20°C).
[b] Wet (imbibition at 4°C or 25°C).
[c] Dry (redrying at 4°C or 25°C).
[d] Descending order of electrolyte conductivity.
[e] Automatic seed analyzer.
[f] $n = 2$.

viability (Fig. 3). The curves in Figure 3 simulate the characteristic loss in seed germination as a function of accelerated aging. As the uptake rate was reduced, the loss of vitality of aged seeds was reduced. Moderate aging (50 h) predisposed the seeds to imbibitional injury, but had little effect on vitality if the seeds were slowly imbibed (Fig. 3). Rapid hydration (1 paper) decreased vitality of 50-h aged seeds to 10%, whereas slow hydration (5 papers) improved germination to 90%. Growth of seedlings was also reduced by imbibition injury (data not shown).

Effect of Priming on Electrolyte Leakage of Aged Seeds. Priming aged seeds reduced the electrolyte conductivity of the steep solution to a fraction of that of the unprimed controls (one-half to one-fifth), as seen in Figure 4. This removal of the predisposition of seed to imbibitional injury was interpreted as repair. Primed seeds were injured less by rapid hydration assuming that electrolyte leakage indicates injury.

Priming increased the vitality of seeds that were germinated on a single layer of germination paper and were therefore prone to imbibitional injury. Twenty-six per cent germination in 'low vigor' seeds was increased to 86% by priming. Also in one experiment, priming increased the vitality of severely accelerated aged seeds from 3 to 23%.

Effect of Priming Temperature on Electrolyte Leakage. The data in Table I indicate that the most effective treatment was to prime 'aged' or 'unaged' seeds at 25°C (rank 4, Table I). A reduction in temperature to 4°C during imbibition (wet) and drying (dry) eliminated this priming effect and resulted in 'primed' seeds which essentially had the same loss of electrolytes as the unprimed controls (see rank 1, Table I). Imbibing at 4°C and drying at 25°C or imbibing at 25°C and drying at 4°C resulting in similar responses in that electrolyte loss was intermediate between the most effective priming and the least effective priming conditions.

Effect of Priming Seed Vigor. Priming improved seed vigor, based on the accelerated aging vigor test (1). The vitality of seeds (% germination) after aging was determined by germinating seeds in the light. The development of Chl was used to determine the number of surviving seeds. Compared to unprimed controls, priming increased seed survival by 140% after 24 h of accelerated aging and 320% after 48 h.

The experiment was a 2 by 2 factorial design consisting of primed and unprimed seeds exposed to 24 or 48 h of accelerated aging. The ratio of primed to unprimed seeds surviving the aging was expressed as an average per cent. Each of the four treatments was replicated twice with 80 seeds in each experimental unit.

DISCUSSION

Woodstock and Tao (7) have shown that accelerated aging predisposed seed embryos to injury during imbibition. This injury was responsible for loss of embryo vitality and reduced the growth of the survivors. Avoidance of imbibitional injury was accomplished by slowing the rate of hydration of excised embryos by use of 30% PEG on the germination paper.

In our studies, the rate of water uptake was controlled by utilizing a matrix of paper without the use of PEG (Fig. 1). This approach removed any confounding due to the absorption or adsorption of chemicals. Unlike the previous report (7), these studies utilized whole seeds rather than embryos. Most of the adverse effects of aging on seed vitality and growth (Fig. 3) were the result of imbibitional injury (Fig. 2) evidenced by electrolyte leakage. A new finding was that the predisposition of aged seeds to imbibitional injury was reversible by priming. The restoration of the seeds performance by priming was temperature dependent (Table I) and interpreted as possible metabolic 'repair' resulting from the turnover of structural components. Priming seeds also more than doubled the survival of seeds in the accelerated aging vigor test (1) which indicates rejuvenation of the entire seed.

A metabolic repair hypothesis is consistent with this report and those of others including Toole and Toole (4), Villiers and Edgecumbe (6). This knowledge will elucidate some of the benefits of seed priming for improved vigor. These findings also have ecological implications as discussed by Villiers (5). Experiments are in progress to further test the metabolic repair hyothesis by a more direct approach.

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